

Architectural changes in larval leaf shelters of *Noctuana haematospila* (Lepidoptera: HesperIIDae) between host plant species with different leaf thicknesses

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ABSTRACT. Insect-food-plant associations have been shown to be influenced by the chemical, physical and nutritional characteristics of plants. We suggest that among insect larvae that use leaf material to build shelters, food-plant use may be constrained by differences in host leaf structure, illuminating a rarely investigated aspect of insect-plant interactions. To explore the potential effects of leaf structure on shelter building behavior in a Neotropical skipper butterfly, we investigated shelter building patterns on two congeneric food-plants that varied in leaf thickness. Shelter architecture varied significantly between hosts, with thicker leaves requiring longer cuts to construct shelters. The relationship between shelter building behavior and leaf structure is discussed in relation to selection pressures on larval shelters and food-plant choice.

KEY WORDS. Behavior; caterpillar; Ecuador; *Rubus*; skipper.

Lepidopteran larvae of at least 18 families construct and occupy shelters or refugia built on or near their food-plant (e.g. DeVRIES 1987, 1997, STEHR 1987, SCOBLE 1992, GREENEY 2009). The architecture and complexity of shelters varies among species, but often involves a precisely executed series of cuts and folds, performed by larvae multiple times throughout their development (e.g. GREENEY & JONES 2003, WEISS *et al.* 2003). This sophisticated and time-consuming behavior has inspired authors to suggest a variety of important roles that shelters may play in larval life history, the most popular being protection from predation (e.g. LOEFFLER 1996, EUBANKS *et al.* 1997, JONES *et al.* 2002). Larval shelter-building behavior can secondarily affect other herbivore-food-plant interactions such as phytochemistry (SANDBERG & BERENBAUM 1989, SAGERS 1992) and may also influence entire arthropod communities by increasing overall arthropod diversity on food-plants (LILL & MARQUIS 2003, 2004). As such, larval shelter construction may yield insight into the ecology and evolution of both insect-food-plant interactions as well as community structure. Of particular interest is the question of whether leaf properties such as thickness or texture may affect the architecture of larval shelters, affecting larval fitness on different food-plants and influencing lepidoptera-food-plant coevolution.

Skipper butterflies (HesperIIDae) construct shelters throughout larval development and show a large amount of interspecific and ontogenetic variation which may be phylo-

genetically informative within this group (GREENEY & JONES 2003, GREENEY 2009). While shelter building is widespread among Neotropical skipper larvae (e.g. MOSS 1949, YOUNG 1985, BURNS & JANZEN 2001), the details of shelter architecture are available for only a few species (e.g. GREENEY & WARREN 2003, 2004, 2009, WEISS *et al.* 2003, GREENEY & YOUNG 2006).

Fifteen years of observations on the shelter building behavior of skipper larvae throughout the Americas suggests that, while final shelter structure is conserved, the details of architecture may vary, even within a species utilizing multiple hosts. We tested this observation by quantifying variation in shelter architecture of first and second larval stadia of a skipper butterfly which feeds on two species of food-plants that differ in leaf thickness.

MATERIAL AND METHODS

Noctuana haematospila (C. Felder & R. Felder, 1867) is a skipper butterfly with well described larval shelters (GREENEY & WARREN 2004), and is distributed from Venezuela to Bolivia in the northern Andes above 1000 m (EVANS 1953). Eggs of *N. haematospila* are laid singly on the dorsal surface of *Rubus* spp. (Rosaceae) host plants. Larvae of all instars construct and occupy shelters on leaves where eggs are laid. Typically, 3-4 shelters are built during larval ontogeny; the first shelter is constructed upon emergence from the egg, and larger subsequent shelters are made as larvae grow, with the second shelter typi-

cally built by late second or early third instars. The first and second larval shelters, termed “Type 3, center-cut shelters” by GREENEY (2009) are round or oval sections of leaf, excised and flipped over onto the dorsal surface of the leaf, folded across a narrow bridge or hinge (Fig. 1). To create such a shelter, a single, circular cut is made in the leaf which curves around to nearly join with its point of origin, leaving an uncut bridge where the shelter folds. Thus, a shorter bridge represents more cutting by the larva. This provides the larvae with a small man-hole-like cover, under which it lives (GREENEY & WARREN 2004). Terminology for describing shelter architecture follows GREENEY & JONES (2003) and GREENEY (2009).

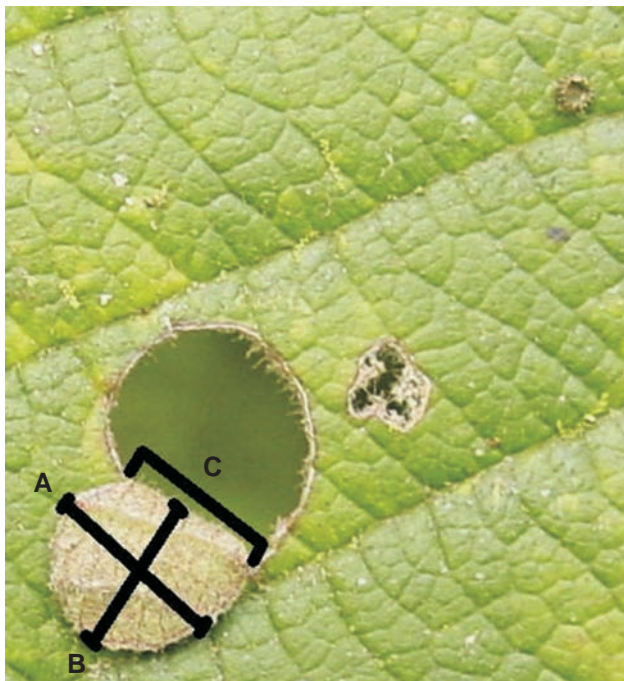


Figure 1. Photograph of first larval shelter of *Noctuana haematospila* in eastern Ecuador. Lines indicate the dimensions recorded for both first and second larval shelters: a) length of shelter lid parallel to the shelter bridge, b) width of the shelter bridge perpendicular to prior measurement, c) bridge width.

We made all observations at the Yanayacu Biological Station and Center for Creative Studies (2100 m a.s.l.) in the Quijos Valley of Ecuador’s NE Napo Province, five kilometers west of the town of Cosanga. Yanayacu Biological Station shares a 2000 ha reserve with Cabañas San Isidro and is bordered by thousands of hectares of primary forest with scattered agricultural communities. For more complete descriptions of habitat in the area, see GREENEY *et al.* (2006) and VALENCIA (1995).

Of the four species of *Rubus* Linnaeus, 1753 known to

occur at the study site, *R. urticifolius* Poiret, 1804 and *R. boliviensis* Focke, 1874 were dominant in preliminary surveys and were the most frequent hosts of *N. haematospila* (GREENEY & WARREN 2004, GREENEY pers. obs.). To assess the relative use of these two *Rubus* spp., we exhaustively searched for early instar shelters of *N. haematospila*, empty and occupied, on all individuals of *R. urticifolius* and *R. boliviensis* at the forest edge along the 5-km road from Yanayacu to Cosanga. Because first instar shelters are more reliably encountered than eggs, we used the presence of first larval shelters to indicate an oviposition event. We collected all leaves encountered with first and second shelters, and made three measures of shelter architecture (Fig. 1) on flattened leaves in the lab using a digital caliper with 0.01 mm precision. To assess the relative thickness of leaves of the two focal *Rubus* spp., we used the same calipers to measure the thickness of 10 fully expanded leaflets from each of 10 plants in the field. We avoided large secondary veins, compressing the caliper arms by hand to measure the thickest portion of the leaf between secondary veins near the leaf edge.

We assessed differences in shelter architecture between food-plant species using t-tests for differences in the mean of each of these three quantified shelter dimensions. We measured patterns of food-plant usage using a Chi-square test for homogeneity of first instar shelter frequency on plant species and a t-test for difference in the mean number of shelters observed on each plant. We compared mean leaf thickness of the two food-plants using a two-tailed t-test after pooling the 100 samples from each plant species.

RESULTS

We surveyed 114 *R. urticifolius* and 87 *R. boliviensis* plants along the road, yielding 149 first instar *N. haematospila* shelters and 86 second shelters. Statistical tests for homogeneity of caterpillar frequency on host plant species based on the average number of first instar shelters per plant within species (t-test result $p < 0.001$) and the number of plants with first shelters ($\chi^2 = 9.736$, $df = 1$, $p < 0.001$) showed *R. boliviensis* was used significantly more often than *R. urticifolia*. Mean leaf thickness (\pm SD) of *R. boliviensis* (0.19 ± 0.03 mm) was significantly less than that of *R. urticifolia* (0.26 ± 0.04 mm) (two-tailed t-test, $p > 0.001$). Dimensions of the excised shelter “lid” (length by width) and the width of the “bridge” where the shelters hinge are presented in table I. While the dimensions of shelter lids were equivalent between food-plant species for first and second shelters, the width of the bridge was significantly larger in first shelters for larvae on *R. boliviensis* (two-tailed t-test, $p < 0.001$), indicating that larvae spent less time (i.e., less cutting) when building first shelters on the thinner-leaved food-plant. Second shelters showed no significant differences (two-tailed t-test, $p = 0.144$) in the width of the bridge between the two hosts, suggesting that larger larvae were less constrained by leaf thickness.

Table I. Measurements of first and second larval shelters of *N. haematospila*, mean, and standard deviation for each of the three measurements of shelter architecture for first two shelters built by larvae. The size of excised leaf material is constant across both host plant species. Width of the bridge where the leaf is folded is significantly greater in shelters built on *R. boliviensis* indicating less cutting was required by larvae on this species.

	Foodplant species	N	Mean shelter width mm (s.d)	Mean shelter length mm (s.d.)	Mean bridge width mm (s.d.)
Shelter 1	<i>R. urticifolia</i>	51	4.029 (0.384)	4.396 (0.448)	1.841 (0.442)*
	<i>R. boliviensis</i>	98	4.139 (0.422)	4.459 (0.417)	2.109 (0.390)*
Shelter 2	<i>R. urticifolia</i>	27	8.632 (1.153)	8.703 (1.068)	3.730 (0.989)
	<i>R. boliviensis</i>	59	8.451 (1.605)	8.810 (1.478)	4.068 (0.962)

DISCUSSION

In an attempt to understand the forces driving food-plant selection and herbivore-food-plant coevolution, biologists have explored a wide variety of physiological, chemical, and evolutionary interactions between many different herbivore-plant taxa (e.g. EHRlich & RAVEN 1965, GILBERT 1971, WOODMAN & FERNANDES 1991, DYER *et al.* 2001, ZALUCKI *et al.* 2001). Plant architecture and leaf morphology, though they have received little attention (MARQUIS 1992), may directly affect the abundance of certain herbivores (HAYSOM & COULSON 1998, MARQUIS *et al.* 2002), as well as intraspecific variation in food-plant use (ALONSO & HERRERA 1996) and larval survival (LOEFFLER 1993). MARQUIS *et al.* (2002) showed that the spatial arrangement of oak leaves had an important effect on the abundance of shelter building larvae, and suggested that increasing distances between adjacent leaves prevented tiny first stadia larvae from tying leaves together. In our investigation of the influence of leaf thickness on shelter architecture, we found *N. haematospila* shelters built by first instars showed significant architectural changes correlated with leaf thickness, but those built by second or third instars showed relatively little change. Thus, like the study of MARQUIS *et al.* (2002), we found that the challenges posed to larvae by leaf thickness were likely most important for early stadia skipper larvae.

As shelter building lepidopteran larvae harness the elastic properties of silk by super-stretching it, and repeatedly spinning numerous lines of silk to move portions of leaves into the desired position (FITZGERALD & CLARK 1994, FITZGERALD *et al.* 1991), it follows that more resistant manipulations will require more time and energy to perform. The bridge portion of the young larval shelters of *N. haematospila* is the hinge on which the shelter lid is folded over to meet the leaf surface. The width of this bridge will have a direct effect on the ease with which this fold may be accomplished: wider bridges will present more resistance to folding than shorter ones. Thus, a larva which cuts more of the leaf, leaving a narrower bridge, will meet with less resistance in folding over the leaf. We found significant differences in the relative width of this bridge between shelters built on two congeneric food-plant species varying in leaf thickness. We suspect this difference is correlated with greater resistance of thicker leaves to folding. As larval shelters may help to protect larvae from pre-

ation and dislodgement (LOEFFLER 1996, EUBANKS *et al.* 1997, JONES *et al.* 2002), longer construction periods may increase exposure to predators (LOEFFLER 1993). Selection, therefore, should favor modifications in shelter building behaviors which minimize this period of exposure. In fact, when comparing larval survivorship on two congeneric plants with varying leaf thickness, LOEFFLER (1993) found that shelter building moth larvae survived better on the thin-leaved host, and postulated that this was due to relative speed with which young larvae could fold thinner leaves, thus reducing their exposure to predators or dislodgement from the plant. Similarly, in the case of *N. haematospila*, host plant choice may affect the amount of labor and time needed to construct larval shelters (more vs. less cutting), and selection should favor females who lay eggs on host plants that allow shelters to be built with minimal exposure time.

We did not test female oviposition preference directly, but the distribution of first stadia larvae among *Rubus* species at our study site provides cautious support for greater oviposition frequency by *N. haematospila* on the plant species which requires less cutting by larvae. To this end, we found clear statistical evidence that more first stadia larvae were found on the thin-leaved *Rubus* species, a pattern which may reflect food-plant preference by ovipositing females, but we did not perform experiments to eliminate other explanations for oviposition patterns. We acknowledge that female host preference may be driven by one or more alternative factors including food-plant phytochemistry, apparency, or predation. Because our observations are consistent with a pattern expected if leaf thickness is important for larval survival, we offer them as an incentive for others to consider the potentially substantial influence of subtle aspects of leaf morphology on food-plant associations in shelter building species.

Shelter building in the Hesperidae is thought to be a fairly conserved character (GREENEY & JONES 2003, GREENEY 2009), and a recent study comparing the micro-architecture of two hesperiids building architecturally similar shelters suggests that even minor modifications to the process of shelter construction may be phylogenetically informative (GREENEY & SHELDON 2008). Our data suggest that understanding the physical characteristics of food-plants, and how these affect shelter construction, may be crucial to understanding the evolution of different shelter types

between lineages. The interaction between plant architecture and leaf morphology, and the construction of herbivore leaf shelters, may affect herbivore-host interactions in several important ways. First, shelter-building herbivores may be constrained by the type of shelter larvae are physically capable of constructing, thus necessitating a shift in shelter architecture before a successful shift in food-plant species can be made. Secondly, as shelter architecture may be affected by food-plant shifts within lineages, we might expect predictable changes in shelter architecture which coincide with ancestral shifts in food-plant species. We suggest that studies of variation in the architectural details of shelter construction, both between and within species feeding on different hosts, will provide additional insight into the evolution of larval shelter construction and food-plant use in shelter building Lepidoptera.

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